Flavor Compounds in Country Cured Hams

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SUMMARY

A study was conducted to identify the flavor compounds in country cured hams. The volatiles were removed from the hams by using steam distillation at reduced pressure and collected on traps cooled with dry ice. The distillate was extracted with ethyl ether and the ether extract was analyzed by gas liquid chromatography. The carbonyl compounds were isolated from the ham lipids by using 2,4-dinitrophenylhydrazine reaction columns. The carbonyl hydrazones were identified by using ultraviolet spectroscopy and thin layer and column chromatography. Free amino and fatty acids were determined by gas liquid chromatography.

Compounds tentatively identified in the volatile fraction included seven carbonyl compounds, six alcohols, six methyl esters and three ethyl esters. The carbonyl compounds found in the ham lipids were C_1-C_{12} n-alkanals, C_6-C_{12} alk-2-enals and C_7, C_9, C_{10}, C_{11}, and C_{12} alk-2,4-dienals. The degree of proteolysis and lipilysis varied among hams produced by different processors and depended upon temperature and length of aging. Total free amino acid contents of the hams ranged from 59 to 153 mg/g, and total fatty acids ranged from 49 to 180 mg/g fat.

INTRODUCTION

Many investigators have studied the flavor components of fresh meats, but only a few have investigated the flavor of cured and aged meats such as country cured ham. It is known that the precursors of meat flavors are water soluble and converted to flavor compounds during cooking of the meat (Batzer *et al.*, 1962; Wood *et al.*, 1957; Hornstein *et al.*, 1960; Macy *et al.*, 1964). It is not known, however, if the same is true for country cured hams.

Initially, workers studied the chemical constituents of country cured ham and related them to the flavor of the product (Kemp *et al.*, 1957; Fields *et al.*, 1955; Blumer, 1954). In 1964, Ockerman *et al.* investigated the volatile flavor compounds in aged ham and found the carbonyl compounds were important in the flavor of the hams and that their levels increased with aging time.

Ockerman (1961) has shown that degradation products of protein and fat increase during aging of hams. These amino acids and fatty acids are known to contribute to flavor of other foods (Day, 1967) and could undoubtedly contribute to the flavor of country cured hams either as flavor compounds or flavor precursors.

Our investigation was undertaken to study the chemical constituents of country cured hams that contribute to the flavor of the product. The chemical entities included volatile components, carbonyl compounds, free amino acids and free fatty acids.

EXPERIMENTAL

Preparation of samples. Fully aged country cured hams were collected from various processors in Missouri, Georgia, North Carolina, Virginia and Kentucky. The hams were cut into cross section slices and the slices separated into fat and lean fractions. The fat was rendered under a stream of nitrogen on a steam bath and used in the analysis of fatty acids and carbonyl compounds. The lean was ground, mixed well and used in analyses for fatty acids and amino acids. The volatile compounds were also collected from the lean fraction.

Vacuum distillation. A 400-g sample of the lean meat was ground in a Waring Blendor with 100 ml of distilled water, and the resulting slurry was placed in a 1-L flask. The volatile compounds were removed by steam distilling the sample for 16 hr at 60°C under a partial pressure of 0.2–0.5 mm Hg in a distillation apparatus similar to that described by Day *et al.* (1960). The volatiles were collected by traps cooled with dry ice-acetone mixtures. The contents of the traps were extracted with 200 ml ethyl ether in a liquid-liquid continuous extractor for 36 hr. The ether solution was concentrated to 2 ml by slowly removing the ether with the aid of a fractionating column head containing an electromagnet set to remove the ether at a ratio of 10:1. The fractionating column was a ¾-in. × 10-ft. column packed with ⅛-in. helices.

Gas liquid chromatography. The volatiles were analyzed by gas chromatography and identified by comparing the retention times of the resulting peaks with those of known compounds. Liquid phases used on the columns were ethylene glycol succinate, Carbowax 20 M, and silicone rubber (GE SE-30). All columns were ¼-in. × 6-ft. and packed with 15% liquid phase on 60–60 mesh diaport W. All of the liquid phases and supports were obtained from Hewlett Packard, F & M Scientific Division. An F & M Model 810 gas chromatograph with dual hydrogen flame detectors was used for the analysis. The operating conditions were: injector temperature, 199°C; detector temperature, 236°C; column temperature, 82°C for 11 min, then increased at 8°/min to 170°C; helium flow rate, 60 ml/min; hydrogen flow rate, 54 ml/min; air flow rate, 333 ml/min.

Free fatty acids. The free fatty acids were isolated from the rendered fat and converted to methyl esters by using ion exchange resin according to the procedure of Bills *et al.* (1963). The free fatty acids were extracted from the lean by the procedure of Hornstein *et al.* (1961). These fatty acids were esterified to methyl esters by using ion exchange resin. The concentrations of all fatty acid methyl esters were determined by gas liquid chromatography (Lillard *et al.*, 1969).

Free amino acids. The gas liquid chromatography procedure by Satterlee *et al.* (1967) was used to determine the amino acid contents of the hams. Fat and moisture contents were determined on the samples by appropriate AOAC procedures (1965). All amino acid data were corrected for the percentages of fat and moisture in the lean portion of the hams.

Carbonyl compounds. The carbonyls of the ham lipids were made into 2,4-dinitrophenylhydrazone derivatives, and these were isolated from the fat by using the procedure of Schwartz *et al.* (1963). The carbonyl compounds were separated into individual compounds by using various combinations of column and thin layer chromatography (Schwartz *et al.*, 1963; Day *et al.*,

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1960; Libbey *et al.*, 1964). The carbonyls were identified by ultraviolet and infrared spectroscopy and thin layer chromatography.

RESULTS AND DISCUSSION

Initially, an experiment was conducted to determine if the precursors of country cured ham flavor were water soluble since previous workers had reported that meat flavor precursors could be extracted with water (Hornstein *et al.*, 1960; Batzer *et al.*, 1960; Wasserman *et al.*, 1965; Zaika *et al.*, 1968). A lyophilized water extract of country cured ham produced a white powdery residue that had a bland salty taste. Upon heating the dry powder in a water bath, an odor similar to roasted meat was observed. A flavor similar to that of meat broth was produced by boiling a water solution of the powder. At no time during the various heat treatments of this powder was a typical country cured ham flavor and aroma detected. This preliminary study indicated that the country cured flavor or its precursors were not water soluble.

Volatile compounds. The distillate collected in the traps during the distillation had an aroma similar to that of country cured ham. This agreed with the finding of Ockerman *et al.* (1964), who obtained typical aroma from vacuum distilled ham.

Approximately 30 peaks were obtained when the ether extract of the volatiles was analyzed on the gas chromatograph. Twenty-three of these were identified by comparing their retention times with known compounds on ethylene glycol succinate, Carbowax 20 M and Silicone rubber columns.

Table 1. Compounds identified in country cured ham volatiles.

Carbonyls	Alcohols	Esters
n-butanal	1-methanol	methyl
n-pentanal	1-propanol	acetate
n-heptanal	1-hexanol	propionate
	1-heptanol	hexanoate
	1-octanol	octanoate
2-butanone	2-octanol	decanoate
2-pentanone		laurate
diacetyl		
2,6-hexanedione		
		ethyl
		formate
		butyrate
		hexanoate

Table 1 lists the volatile compounds identified in the ham. Most of these compounds are not unique for country cured ham, having been found in the volatiles of cooked meats (Hornstein *et al.*, 1964; Ockerman *et al.*, 1964). Ockerman *et al.* (1964) also found n-butanal, n-pentanal, 2-butanone, 2-pentanone, and diacetyl in the volatile fraction of country cured ham. Some of the more volatile carbonyls reported by Ockerman *et al.* (1964) were not found during this investigation and could be due to the treatment of the distillate before gas chromatography analysis. Ockerman *et al.* (1964) converted the carbonyls in the distillate to 2,4-dinitrophenylhydrazones and regenerated them with alpha-ketoglutaric acid before injecting them into the gas chromatograph. Also, their procedure did not enable them to detect any alcohols or esters in the ham volatiles.

Many of the alcohols listed in Table 1 were identified in cooked chicken by Minor *et al.* (1965). Important precursors of these alcohols could be lipids (Keeney, 1962). Very few of the esters isolated from aged hams have been reported in studies of other meat volatiles. Esterification of the large number of free fatty acids present in aged hams may be the source of these esters.

Carbonyl compounds. The carbonyl compounds identified in the lipids of country cured hams are listed in Table 2. All compounds could not be detected in every sample. Some of the compounds were found in small concentrations and may have been lost during the isolation of carbonyls from the samples. Also, the types of compounds produced by lipid autoxidation depend upon the fatty acid composition of the lipids, which could be influenced by the diet of the hogs (Craig *et al.*, 1964). With the exception of n-heptanal n-dodecanal, dodec-2-enal, and undeca-2,4-dienal, all the compounds listed in Table 2 have been identified as oxidation products of pork fat (Ellis *et al.*, 1961; Gaddis *et al.*, 1957). These compounds were found, however, in autoxidation studies of other lipids (Hoffmann, 1962). Different techniques used to isolate the carbonyls from the fats could account for the differences obtained by various workers.

Table 2. Carbonyl compounds in country cured ham lipids.

alkanals	alk-2-enals	alk-2,4-dienals
methanal	hex-2-enal	hepta-2-,4-dienal
ethanal	hept-2-enal	nona-2,6-dienal
propanal	oct-2-enal	deca-2,4-dienal
n-butanal	non-2-enal	undeca-2,4-dienal
n-pentanal	dec-2-enal	dodeca-2,4-dienal
n-hexanal	undec-2-enal	
n-heptanal	dodec-2-enal	
n-octanal		
n-nonanal		
n-decanal		
n-undecanal		
n-dodecanal		

Lipid and protein degradation products. The large amount of lipid and protein degradation that occurred during aging of the hams agrees with the findings of others (Kemp *et al.*, 1957; Ockerman, 1961; Craig *et al.*, 1964). Large variations in the concentrations of total free fatty and amino acids were found in hams produced in various plants in the United States (Tables 3, 4, 5). This was expected because the hams were aged for different periods of time and at different temperatures. Also, some of the processors controlled humidity while other firms cured the hams in natural humidity.

Hams collected from Missouri, Georgia and Kentucky contained high concentrations of free fatty acids in the fat surrounding the lean. (The processors in Georgia and Kentucky aged their hams at higher temperatures than did the other firms, while the hams from the plant in Missouri were aged longer.) This was not true, however, for the free fatty acids of the lean. Hams collected in North Carolina and Virginia had more free fatty acids in the lean meat than did other hams. This could be due to the reaction between fatty acids and other constituents of the lean in the hams aged at higher temperatures or for longer periods.

The relative composition of fatty acids varied from sample to sample.

Table 3. Free fatty acids in commercial country cured hams.

	Origin of Hams					
	Iowa (fresh)	Ky.	N.C.	Va.	Ga.	Mo.
Fatty acid no.	mg/g fat					
8:0	T[1]	.05	T	T	T	T
10:0	T	.14	.09	.09	T	.12
12:0	.03	.08	.03	.04	T	.09
14:0	.05	1.54	1.55	.64	3.37	2.06
16:0	1.18	12.93	10.78	10.94	23.80	20.58
16:1	.18	2.27	1.56	2.46	5.76	2.98
18:0	1.30	10.06	9.27	4.23	27.72	22.84
18:1	3.88	29.58	32.18	20.92	77.68	48.89
18:2	1.44	14.45	1.21	7.75	27.86	15.51
18:3	.07	1.89	.69	.49	4.38	2.36
20:0	.06	2.29	1.07	.76	4.16	1.06
20:1	.04	.64	.53	.86	2.06	1.17
20:2	T	.56	.42	.39	.85	T
20:4	T	T	T	T	2.39	5.13
Total	8.23	76.48	59.38	49.57	180.03	122.79

[1] T = Trace.

The lipid composition of pork is affected by the feed the hogs consume (Craig *et al.*, 1964). The lipid composition of the pigs' diets was likely different and would account for the difference in fatty acid composition of the hams. The degree of proteolysis that occurred in the hams also varied between hams. As with lipolysis, this can be related to the temperature and length of aging of the hams (Table 5).

Flavor of country cured ham. Results of this study indicated that the flavor and flavor precursors of country cured hams were not water soluble. An aroma typical to country cured hams could be obtained by steam distillation at 60°C under reduced pressure. A raw country cured ham has an aroma and flavor quite different from fresh pork. This indicates that the country cured flavor is produced during aging of the hams. When the hams are cooked, this aroma and flavor is intensified, thus indicating that the heating process accelerates some of the reactions involved in producing the country cured flavor. The degradation products of the ham lipids are important flavor compounds and undoubtedly include precursors that are converted to flavor compounds when heated. The carbonyl compounds produced by autoxidation of the lipids contribute to the flavor of the hams. Free fatty and amino acids contribute to the flavor of many foods (Day, 1967) and must be important in the flavor of country hams.

Although many chemical components were found in country cured hams, no attempt was made in this investigation to correlate any single compound or groups of compounds to the flavor of country cured hams. This is an important part of flavor research and should be investigated in future work.

REFERENCES

AOAC. 1965. Official methods of analysis of the Association of Official Analytical Chemists. 10th ed. Washington, D. C.

Batzer, O. F., Santoro, A. T., Tan, M. C., Landmann, W. A. and Schweigert, B. S. 1960. Precursors of beef flavor. *Agri. and Food Chem.* **8**, 498.

Batzer, O. F., Santoro, T. A. and Landmann, W. A. 1962. Identification of some beef flavor precursors. *Agri. and Food Chem.* **10**, 94.

Bills, D. D., Khatri, L. L. and Day, E. A. 1963. Method for the determination of the free fatty acids in milk fat. *J. Dairy Sci.* **46**, 1342.

Table 4. Free fatty acids in the lean tissue of commercial country cured hams.

	Origin of Hams					
	Iowa (fresh)	Ky.	N.C.	Va.	Ga.	Mo.
Fatty acid no.	mg/g fat[1]					
8:0	T[2]	T	T	.17	T	.16
10:0	.16	.07	.14	.07	.06	.10
12:0	.16	.03	.31	.02	.05	.04
14:0	.95	.50	6.93	.05	.21	2.07
16:0	5.83	3.89	19.22	14.44	7.64	10.67
16:1	T	1.06	2.17	1.10	.67	1.66
18:0	8.75	10.95	27.41	19.65	13.02	15.60
18:1	5.88	13.24	31.52	25.79	12.24	21.57
18:2	17.02	14.15	20.21	36.83	20.60	20.68
18:3	T	.02	.11	.24	1.23	.20
20:0	T	.03	.77	1.02	5.79	.97
20:1	T	.02	.26	1.18	T	.87
20:4	4.92	4.39	2.40	9.14	T	7.79
Total	43.67	48.35	111.45	109.59	61.51	82.27

[1] Corrected for fat and moisture content.
[2] T = Trace.

Table 5. Free amino acids in commercial country cured hams.

	Origin of Hams					
	Iowa (fresh)	Ky.	N.C.	Va.	Ga.	Mo.
Amino acid	mg/g[1]					
alanine	.62	9.36	4.22	3.25	1.59	12.75
valine	.78	8.91	2.83	3.07	5.86	11.83
isoleucine	.61	3.08	2.01	2.30	4.96	8.23
glycine	.50	2.52	1.78	4.78	4.15	11.16
threonine + leucine	1.39	11.53	4.89	5.12	7.07	20.39
proline + serine	—	2.62	1.06	1.67	2.63	5.01
hydroxyproline	—	5.71	.42	1.63	—	4.22
methionine	—	3.78	.83	1.67	5.03	12.64
aspartic acid + phenylalanine	1.28	18.05	2.61	6.10	1.55	27.81
glutamic acid	.40	12.77	4.21	3.68	3.72	21.19
tyrosine	1.12	15.45	34.98	31.81	38.24	18.63
lysine	—	—	—	8.98	—	—
tryptophan	—	—	—	—	—	—
Total	6.78	93.78	59.84	74.06	74.80	153.76

[1] Corrected for moisture and fat content.

Blumer, T. N. 1954. Chemical compounds associated with aged ham flavor. Ph.D. thesis, Michigan State University, East Lansing, Michigan.

Craig, H. B., Blumer, T. N., Clawson, A. J. and Smart, W. W. O., Jr. 1964. Chemical, physical, and organoleptic evaluations of aged country-style hams from hogs fed rations with and without peanut oil. *J. Animal Sci.* **23**, 956.

Day, E. A. and Lillard, D. A. 1960. Autoxidation of milk lipids. 1. Identification of volatile monocarbonyl compounds from autoxidized milk fat. *J. Dairy Sci.* **43**, 585.

Day, E. A. 1967. Cheese flavor. In "Chemistry and Physiology of Flavors," eds. Schultz, H. W., Day, E. A. and Libbey, L. M. pp. 331–362. Avi Publ. Co., Inc., Westport, Conn.

Ellis, R., Gaddis, A. M. and Currie, G. T. 1961. Carbonyls in oxidizing fat. IV. The role of various fatty acid components in carbonyl generation. *J. Food Sci.* **26**, 131.

Fields, M. D., Dunker, C. F. and Swift, C. E. 1955. The effects of storage on the composition and nutritive properties of farm-style hams. *Food Technol.* **9**, 491.

Gaddis, A .M. and Ellis, R. 1957. Volatile saturated aldehydes in rancid fat. *Science* **126**, 745.

Hoffmann, G. 1962. Vegetable oils. In "Symposium on Foods: Lipids and Their Oxidation," eds. Schultz, H. W., Day, E. A. and Sinnhuber, R. O. pp. 215–229. Avi Publ. Co., Inc., Westport, Connecticut.

Hornstein, I. and Crowe, P. F. 1960. Flavor studies on beef and pork. *Agri. and Food Chem.* **8**, 494.

Hornstein, I., Crowe, P. F. and Heimberg, M. J. 1961. Fatty acid composition of meat tissue lipids. *J. Food Sci.* **26**, 581–586.

Hornstein, I. and Crowe, P. F. 1964. Meat flavor. A review. *J. Gas Chrom.* **2**, 128.

Keeney, M. 1962. Secondary degradation products. In "Symposium on Foods: Lipids and their Oxidation," eds. Schultz, H. W., Day, E. A. and Sinnhuber, R. O. pp. 70–89. Avi Publ. Co., Inc., Westport, Connecticut.

Kemp, J. D., McCampbell, H. C. and Grainger, R. B. 1957. Procedure for sampling of ham fat and characteristics of hams and fat during aging. *Food Technol.* **11**, 321.

Libbey, L. M. and Day, E. A. 1964. Reverse phase thin-layer chromatography of n-alkanols and n-alkan-2-ones. *J. Chromatog.* **4**, 273.

Lillard, D. A. and Ayres, J. C. 1969. The effects of using mold cultures in the production of country cured hams. *J. Food Sci.* (in press).

Macy, R. L., Jr., Nauman, H. D. and Bailey, M. E. 1964. Water soluble flavor and odor precursors of meat. 1. Qualitative study of certain amino acids, carbohydrates, non-amino acid nitrogen compounds and phosphoric acid esters of beef, pork, and lamb. *J. Food Sci.* **29**, 136.

Minor, L. J., Pearson, A. M., Dawson, L. E. and Schweigert, B. S. 1965. Chicken flavor: The identification of some chemical components and the importance of sulphur compounds in

the cooked volatile fraction. *J. Food Sci.* **30**, 686.
Ockerman, H. W. 1961. Separation and identification of volatile compounds in dry-cured hams. Ph.D. Thesis. North Carolina State College, Raleigh. N. C.
Ockerman, H. W., Blumer, T. N. and Craig, H. B. 1964. Volatile chemical compounds in dry-cured hams. *J. Food Sci.* **29**, 123.
Satterlee, L. D. and Lillard, D. A. 1967. A procedure for the gas chromatographic analysis of free amino acids in meats. *J. Food Sci.* **32**, 682.
Schwartz, D. P., Haller, H. S. and Keeney, M. 1963. Direct quantitative isolation of monocarbonyl compounds from fat and oils. *Anal. Chem.* **35**, 2191.
Wasserman, A. E. and Gray, N. 1965. Meat flavor. 1. Fractionation of water-soluble flavor precursors of beef. *J. Food Sci.* **30**, 801.
Wood, T. and Bender, A. E. 1957. Analysis of tissue constituents. Commercial ox-muscle extract. *Biochem. J.* **67**, 366.
Zaika, L. L., Wasserman, A. E., Monk, C. A. and Saley, J. 1968. Meat flavor. 2. Procedures for the separation of water-soluble beef aroma precursors. *J. Food Sci.* **33**, 53.
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Holding Raw Fish (Red Hake) in Isopropyl Alcohol for FPC Production

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SUMMARY

Whole, ground, red hake were stored in 91% isopropyl alcohol for 1, 2, 4, 7 and 11 days at room temperature. After each storage period the fish were processed into FPC and analyzed for proximate composition, amino acids, available lysine and the nutritive quality of the protein. The results indicated that small losses in nitrogen occurred, thus decreasing yield. First-stage solids were analyzed for total bacterial content and did not show any increase with storage time. The nutritive quality was not impaired and the PER's were found to be significantly higher than casein.

Analysis of the storage alcohol showed increases in soluble nitrogen with storage time, which appear to be indicative of enzymatic proteolysis and solution of nitrogenous material.

INTRODUCTION

A prerequisite to the manufacture of fish protein concentrate is the prime quality of the raw material. Preferably, the fish should be handled as at a day or overnight fishery. The fish should be preserved by some suitable means from ship to shore and then processed immediately.

Such ideal procedures, however, are not always possible. Fishing boats may have to make longer trips than anticipated; preservation procedures, such as holding the fish in ice, can be very costly and cumbersome; oftentimes the catch of fish exceeds the capacity of the plant and must be held; breakdowns in the plant may interfere with processing schedules. Provision must be made, therefore, for the storage of fish in a manner that maintains quality. In considering the Bureau of Commercial Fisheries process for the manufacture of fish protein concentrate, the suggestion has been made that the raw, ground fish be stored in isopropyl alcohol (IPA) on the boat or in the plant.

Isopropyl alcohol has been shown to act as a good preservative for cod fish livers (Guttmann *et al.*, 1957; Jangaard *et al.*, 1965). Furthermore, the solvent was found to inhibit lipolytic enzymes and bacterial growth in fillet waste for periods up to 3 months (Guttmann *et al.*, 1967). No results, however, have been reported relating storage of whole lean fish in IPA, the raw material for FPC production, to yield, composition, and nutritive quality of final product.

Therefore, a study was initiated to determine the effect of IPA storage of whole fish on the FPC prepared from it.

EXPERIMENTAL

Processing methods. One hundred and twenty pounds of red hake (*Urophycis chuss*) were caught off the coast of Rhode Island, iced on the vessel, frozen at the dock, and then shipped to this laboratory. The frozen fish were ground through a Hobart grinder having a 1/8-in. end plate and then divided into six lots, each weighing 20 pounds. Each lot was then placed in a battery jar containing 91% v/v IPA at a w/w ratio of 1:1.3 (fish–azeotrope IPA ratio) and stirred continuously for 5 min at high speed using an air-driven stirrer.

The battery jars were then covered with aluminum foil and held at room temperature (22°C ± 2°) for 1, 2, 4, 7 and 11 days. Each lot was processed into FPC in the manner described by Brown *et al.* (1969).

Analytical methods. *Chemical analyses.* Chemical analyses of the FPC products for crude protein, ash, and volatiles were performed in accordance with AOAC techniques. Total lipids were analyzed by the method of Smith *et al.* (1964). Amino acids were determined on an acid hydrolysate (Spackman *et al.*, 1958) using a Beckman-Spinco AutoAnalyzer. Available lysine was measured by the method of Carpenter (1960) and tryptophan in accordance with Spies (1949). Total nitrogen and lipid analyses were also obtained on aliquots of filtered samples of the storage alcohol. This alcohol is considered in this report as the first miscella.

Bacteriological analyses. Total plate counts were determined on the raw material prior to storage. In addition, the material from the battery jars for each storage period was centrifuged and total plate counts were obtained for these samples. Duplicate samples were taken and plated in triplicate, using Difco plate count agar. The plates were incubated at 35°C for 48 hr or 20°C for 5 days.

Nutritive evaluation. Samples of FPC prepared from IPA-stored raw fish were fed *ad libitum* to male albino